Iridoid Glycosides from *Globularia davisiana*

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From the ethanolic extract of the aerial parts of *Globularia davisiana*, a new iridoid glycoside, davisioside (1), was isolated. Davisioside (1) comprises a rare iridoid aglycone structure with a saturated double bond between C-3 and C-4. Nine known iridoid glycosides, asperuloside (2), alpinoside (3), geniposide (4), globularin (5), globularicisin (6), 10-*O*-benzoylcatalpol (7), lytanthosalin (8), melampyroside (9), agnuside (10), and three known phenylethanoid glycosides, verbascoside, isoacteoside and leucosceptoside A were also isolated and characterized. The structures of the isolates were established by spectroscopic methods (one-dimensional (1D)- and two-dimensional (2D)-NMR, MS).

Key words Iridoid glycoside; phenylethanoid glycoside; *Globularia davisiana*; Globulariaceae

The genus *Globularia* (Globulariaceae) is represented by eight species in the flora of Turkey.[1)] In Anatolian folk medicine, *G. alypum* is used as a diuretic, laxative, stomachic and tonic,[2)] while *G. trichosantha* is utilized for the treatment of hemorrhoids.[3)] In our previous papers, we reported phenylethanoid glycosides, iridoid and bisiridoid glycosides from *G. trichosantha*.[4,5)] In the continuation of chemical studies of Turkish *Globularia* species, we have investigated an endemic species, *G. davisiana*. We herein present the isolation and structure elucidation of davisioside (**1**), a new iridoid glycoside with a saturated $\Delta^{3,4}$ obtained from the aerial parts of *G. davisiana*.

Davisioside (**1**) was obtained as an amorphous powder. The molecular formula, $C_{22}H_{28}O_{10}$, requiring nine degrees of unsaturation, was deduced by a combination of electrospray ionization mass spectroscopy (ESI-MS) (*m/z* 475, $[M+Na]^+$), high resolution (HR)-FAB-MS (*m/z* 435.1675, $[M-H_2O+H]^-$) and ^{13}C-NMR data. Compound **1** exhibited UV maxima at 229 and 274 nm. The IR spectrum suggested the presence of hydroxyl (3421 cm^{-1}), ester carbonyl (1715 cm^{-1}), olefinic (1654 cm^{-1}) and aromatic (1508, 1451 cm^{-1}) functionalities. The ^{1}H-NMR spectrum (Table 1) contained signals due to an olefinic proton (δ_H 5.86), an acetal proton (δ_H 4.96), an oxygenated methine proton (δ_H 4.56), two oxymethylenes (δ_H 3.99 and 3.58; δ_H 5.08 and 4.94), two methines (δ_H 2.43, 2.86) and two diastereopic protons of a methylene (δ_H 1.66, 1.81). Additional aromatic proton signals at δ_H 8.06 (2H), 7.62 (1H) and 7.49 (2H) and the corresponding carbon resonances (Table 1) were typical of a monosubstituted phenyl moiety. The signals in the region of δ_H 3.20—3.80 (6H) accompanied by an anomeric proton resonance at δ_H 4.60 (d, J=7.8 Hz) supported that **1** contained a β-glucopyranosyl unit. The ^{13}C-NMR spectrum of 1 displayed 22 signals, six of which could be attributed to a β-glucopyranosyl unit, while seven of which were ascribed to a benzoic acid moiety. All the remaining ^{13}C signals, established by distortionless enhancement by polarization transfer (DEPT)-90, DEPT-135, gradient heteronuclear single quantum coherence (gHSQC) and gradient heteronuclear multiple bond correlation (gHMBC) experiments, were assignable to a dihydroaucubin type iridoid core.[6)] The double quantum filtered correlation spectroscopy (DQF-COSY) spectrum of **1** revealed that two methylene and five methine protons of the aglycone existed as one proton spin system (Fig. 1). The proton sequence started with the acetal proton, H-1, which showed coupling with H-9. The latter proton was further coupled to H-5. Additional scalar couplings were obtained between H-5/H_2-4 and H_2-4/H_2-3. In the other direction, H-5 correlated to a β-hydroxy bearing proton (δ_H 4.56, H-6), which further coupled to the olefinic proton, H-7. The absence of any other homonuclear couplings observed for H-7 was indicative of C-8 being fully substituted. The gHMBC spectrum (Table 1) allowed assignment of the remainder of the aglycone, where the expected long-range couplings for dihydroaucubin were observed (Table 1). However, the pro-

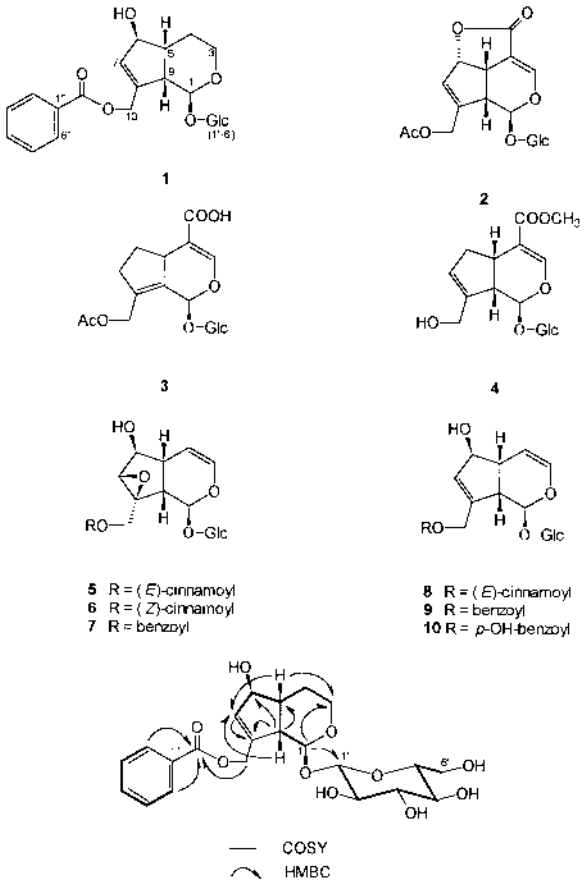

Fig. 1. COSY and Some Selected HMBC Correlations for **1**

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Table 1. ^{13}C- (100 MHz), ^{1}H-NMR (400 MHz) and Complete gHMBC (J=8 Hz) Data for Davisioside (**1**) in CD_3OD[a)]

C/H	δ_C ppm	DEPT	δ_H ppm, J (Hz)	HMBC (H→C)
1	95.5	CH	4.96 d (6.0)	C-1′, C-3
3α	61.7	CH_2	3.99 m	C-1, C-5
3β			3.58 m	
4α	25.3	CH_2	1.66 m	
4β			1.81 m	
5	46.1	CH	2.43 m	C-3, C-4, C-6, C-9
6	79.3	CH	4.56 br d (5.5)	C-8
7	132.2	CH	5.86 br s	C-5, C-6, C-8, C-9, C-10
8	143.8	C		
9	48.6	CH	2.86 t (6.0)	C-1, C-5, C-6, C-7, C-8
10	63.9	CH_2	5.08 d (14.7)	C-7, C-8, C=O
			4.94 d (14.7)	
1′	99.6	CH	4.60 d (7.8)	C-1
2′	74.9	CH	3.20 dd (7.8, 8.9)	C-1′, C-3′
3′	78.1	CH	3.36 t (8.9)	C-2′
4′	71.6	CH	3.26[b)]	C-3′, C-5′
5′	78.2	CH	3.25[b)]	C-6′
6′	62.8	CH_2	3.80 dd (11.9, 1.6)	C-5′
			3.64 dd (11.9, 5.2)	
1″	131.3	C		
2″	130.6	CH	8.06 dd (7.4, 1.3)	C=O, C-1″
3″	129.6	CH	7.49 t (7.4)	C-1″
4″	134.4	CH	7.62 tt (7.4, 1.3)	C-2″, C-6″
5″	129.6	CH	7.49 t (7.4)	C-1″
6″	130.6	CH	8.06 dd (7.4, 1.3)	C=O, C-1″
C=O	167.7	C		

a) All proton and carbon assignments are based on 2D NMR (DQF-COSY, gHSQC and gHMBC) experiments. *b*) Signal patterns are unclear due to overlapping.

ton signals assigned to H_2-10 appeared to be deshielded. This finding, together with the gHMBC correlation between H_2-10 and the carbonyl carbon of the benzoic acid suggested C-10 to be the site of acylation. The gHMBC correlations between H-1 and C-1′ and *vice versa*, indicated that the β-glucopyranosyl unit was attached at the usual position, C-1. To prove the relative stereochemistry of the chiral centers in **1**, a two-dimensional nuclear Overhauser effect spectroscopy (2D NOESY) experiment was performed. NOe cross-peaks of significant intensity between H-9/H-5, H-5/H-4β, and H-4β/H-3β indicated these protons to lie on the same side (β) of the molecule. Contrary, prominent nOe correlations were observed between H-1/H-3α and H-3α/H-4α and H-4α/H-6α. Therefore, the secondary alcohol functions at C-1 and C-6 had to be in the β position. These data also confirmed the *cis* fusion of the cyclopentan and pyran rings as expected. Consequently, the structure of **1** was established as 10-*O*-benzoyl-3,4-dihydroaucubin.

The NMR and MS data for asperuloside (**2**),[7)] alpinoside (**3**),[8)] geniposide (**4**),[9)] globularin (**5**),[10)] globularicisin (**6**),[10)] 10-*O*-benzoylcatalpol (**7**),[11)] lytanthosalin (**8**),[12)] melampyroside (**9**),[13)] agnuside (**10**),[14,15)] as well as verbascoside,[16)] isoacteoside,[17)] and leucosceptoside A[18)] were identical with published data. All isolates were tested for their radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH).[19,20)] Only the phenylethanoid glycosides were found to possess antioxidant property (yellow-on-purple spot).

Davisioside (**1**) represents a rare iridoid skeleton lacking the double bond between C-3 and C-4. Globularidin, isolated from *Globularia alypum*,[21)] was the first reported iridoid glycoside with such an aglycone. Globularidin has also been isolated from *G. trichosantha*.[5)] Therefore, it is possible that this type of iridoids are common in the family Globulariaceae.

Experimental

Optical rotation was measured on a JASCO DIP-370 digital polarimeter using a sodium lamp operating at 589 nm. UV spectrum was recorded on a Shimadzu UV-160A spectrophotometer. IR spectrum (KBr) was measured on a Perkin Elmer 2000 FT-IR spectrometer. NMR measurements in CD_3OD were performed on a Varian unit, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Negative- and positive-mode ESI-MS were recorded on a Finnigan TSQ 7000 instrument. FAB-MS measurements were performed on a Finnigan MAT95 spectrometer. TLC analyses were carried out on silica gel 60 F_{254} precoated plates (Merck, Darmstadt); detection by 1% vanillin/H_2SO_4. For medium pressure liquid chromatography (MPLC) separations, a Lewa M5 pump, a LKB 17000 Minirac fraction collector, a Rheodyne injector, and a Büchi column (column dimensions 2.6×46 cm, and 1.8×35 cm) were used. Silica gel 60 (0.063—0.200 mm; Merck, Darmstadt) was used for open column chromatography (CC) and vacuum liquid chromatography (VLC). MPLC separations were performed over LiChroprep C-18 (Merck) material.

Plant Material *G. davisiana* O. Schwarz was collected from Antalya, Beldibi, in South Anatolia, Turkey, in June 2000. The voucher specimen (HUEF 00286) has been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and Isolation The air-dried aerial parts (250 g) of *G. davisiana* were extracted with EtOH (2×2 l) at 45 °C. The combined ethanolic extracts were dried *in vacuo* (53 g, yield 21%). The crude extract was dissolved in H_2O and partitioned between CH_2Cl_2 and *n*-BuOH. An aliquot (21 g) of the lyophilized *n*-BuOH phase (30 g) was fractionated over SiO_2 (VLC). Employment of CH_2Cl_2–MeOH–H_2O mixtures (90 : 10 : 1 to 60 : 40 : 4) afforded nine main fractions, A-I. Fraction B (2.93 g) was subjected to reverse phase (RP)-MPLC using step gradient MeOH in H_2O (30—100%) to yield **8** (150 mg) and four additional fractions, B_1—B_4. Fraction B_2 (80 mg) was rechromatographed on SiO_2 eluting with CH_2Cl_2–MeOH–H_2O (90 : 10 : 1) to give **6** (30 mg). Fraction B_4 (1.8 g) was also fractionated by RP-MPLC using *iso*-PrOH gradients in H_2O (10—30%) to yield **9** (40 mg) and fr B_{4b} (1.0 g), separation of which was carried out by SiO_2 CC to give **5** (67 mg). Fraction D (2.5 g) likewise was subjected to RP-MPLC (20—50% MeOH) to yield compounds **3** (7 mg), **2** (18 mg), in addition to frs. D_3—D_{10}. Purification of fr. D_4 (106 mg) and fr. D_6 (227 mg) by SiO_2 CC furnished **4** (63 mg) and **7** (103 mg), respectively. Leucosceptoside A (13 mg) was also purified by SiO_2 CC from fr. D_8 (97 mg) by employing an isocratic elution of $CHCl_3$–MeOH–H_2O (80 : 20 : 2). Fraction D_9 (152 mg) was also subjected to gradient CC over SiO_2 ($CHCl_3$–MeOH–H_2O, 85 : 10 : 1 to 80 : 20 : 1) to yield frs. D_{9a} (40 mg) and D_{9b} (52 mg). Repeated chromatography of fr. D_{9a} on a SiO_2 column using $CHCl_3$–MeOH–H_2O (80 : 15 : 1) gave **1** (24 mg). Melampyroside (**9**, 134 mg) was obtained from fr. D_{10} (370 mg) by SiO_2 CC, employing $CHCl_3$–MeOH–H_2O (90 : 10 : 1 to 80 : 20 : 2) as mobile phase. Fraction E (3.3 g) was subjected to RP-MPLC using stepwise gradients of MeOH (5—70%) in H_2O and yielded seven main fractions, E_1—E_7. Fraction E_4 (505 mg) was rechromatographed on SiO_2 ($CHCl_3$–MeOH–H_2O, 80 : 20 : 2 to 61 : 32 : 7) to give frs. E_{4a} (20 mg) and E_{4b} (313 mg). Repeated CC of fr. E_{4a} over SiO_2 afforded **10** (6 mg). Fraction E_{4b} was further purified by RP-MPLC using H_2O–MeOH mixtures (10—40% MeOH) to yield verbascoside (55 mg). An aliquot (47 mg) of the fr. E_6 (131 mg) was applied to a SiO_2 column. Elution with $CHCl_3$–MeOH–H_2O (80 : 15 : 1 to 80 : 20 : 2) yielded isoacteoside (8 mg).

Davisioside (**1**): Amorphous white powder, $[\alpha]_D$ −69° (c=0.48, MeOH); ESI-MS m/z 475 $[M+Na]^+$; FAB-MS m/z: 435 $[M-H_2O+H]^+$ (5), 273 (16), 255 (87), 185 (63), 149 (65), 93 (100); HR-FAB-MS m/z: 435.1675, Calcd for $C_{22}H_{27}O_9$ 435.1655; UV λ_{max} (MeOH, nm): 229, 274; IR ν_{max} (KBr, cm^{-1}): 3421, 1715, 1654, 1508, 1451; ^{1}H-NMR (CD_3OD, 400 MHz) Table 1; ^{13}C-NMR (CD_3OD, 100 MHz) Table 1.

Reduction of DPPH Radical Methanolic solutions (0.1%) of all isolates were chromatographed on a Si gel TLC plate using $CHCl_3$–MeOH–H_2O (61 : 32 : 7) solvent system. After drying, TLC plates were sprayed with a 0.2% DPPH (Fluka) solution in MeOH. Compounds showing a yellow-on-purple spot were regarded as antioxidant.[20)]

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